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SUBSURFACE BIOLOGICAL ACTIVITY IN RELATION TO GROUND WATER POLLUTION

William J. Dunlap, et al

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SUBSURFACE BIOLOGICAL ACTIVITY IN RELATION TO GROUND WATER POLLUTION

By

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ABSTRACT

Biological activity occurring in subsurface regions below the soil zone may be of considerable importance in determining the fate and effect of pollutants in ground water, but this possibility has received little previous attention. This paper comprises a discussion of subsurface biological activity in regard to ground-water pollution as reflected by available literature references. The subsurface environment is discussed in terms of factors likely to be of greatest significance in regard to the development of biological systems, and previous investigations of subsurface microbial activity are reviewed. Available information indicates the presence in the upper continental crust of the earth of numerous regions, particularly those of sedimentary origin, which are probably suitable habitats for many microbial species. Previous investigations of subsurface microbial activity clearly show the presence of diverse microbial populations in many subsurface regions below the soil zone. Hence, microbial activity appears both possible and probable in most subsurface regions of importance in regard to ground water. Further elucidation of the extent and nature of microbial activity in subsurface regions is needed in developing methods for predicting the impact on ground-water quality of pollutants released into the earth's crust.

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SECTION I

CONCLUSIONS

Currently available information permits the following conclusions concerning subsurface biological activity in relation to ground-water pollution.

1. The upper continental crust of the earth comprises a highly structured and complex environment, characterized by limited open space and a myriad of environmental possibilities. In most subsurface regions, particularly those of sedimentary origin, these environmental possibilities do not appear so severe as to preclude microbial activity until depths are attained where temperatures exceed microbial tolerance levels. These depths are apparently in excess of 2,000 m in most regions.

2. Field investigations pertaining to subsurface microbial activity are difficult due to the inaccessability of subsurface regions and the consequent difficulty in obtaining uncontaminated, representative samples from such regions. Nevertheless, a number of credible investigations reported in the literature clearly show the presence of diverse microbial populations in many subsurface regions lying below the soil zone.

3. Available information indicates strongly that microbial activity is both possible and probable in most subsurface regions below the soil zone which are of importance in regard to ground water. Hence, possible biochemical alteration of pollutants in these regions, as well as possible effects of pollutants on subsurface ecosystems, must be considered in attempting to control pollution of ground water.

4. Investigations to further elucidate the extent and nature of microbial activity in subsurface regions below the soil zone should be included in general studies pertaining to the movement, fate, and effect of pollutants in subsurface waters if these studies

are to provide an effective basis for assessing the probable impact of human activities on the ground-water resource and for regulating and controlling the conduct of such activities to minimize the threat to ground-water quality.

SECTION II

RECOMMENDATIONS

It is recommended that investigations be instigated to further elucidate the potential role in ground-water pollution and pollution control of microbial activity in subsurface regions lying below the soil zone. These investigations should be an integral part of more extensive studies pertaining to the movement, fate, and effect of pollutants in subsurface waters.

The extent and nature of native microbial activity in the zone of aeration below the soil zone and in the upper regions of the zone of saturation should be systematically investigated. These investigations should attempt to establish relationships between native microbial populations and subsurface environmental characteristics, particularly geology and oxidation-reduction conditions.

Microbial activity and microbe-pollutant interactions in subsurface regions receiving pollutants should be subjected to thorough, integrated investigations. In-situ studies should be employed to the fullest possible extent. These studies should seek to correlate native microbial populations, environmental situations (geology, oxygen and nutrient availability, pressure, temperature, etc.), nature of pollutants, and microbial populations after introduction of pollutants with the fate and effect of pollutants.

The ultimate objective of the recommended investigations should be the development of information needed for defining the capacity of subsurface regions as pollutant receptors and, hence, for predicting the probable impact on ground water of activities entailing the release of pollutants into the earth's crust.

SECTION III

INTRODUCTION

Ground water--water beneath the earth's surface--is a little-appreciated but valuable national resource. Approximately 20 percent of the total quantity of water currently used for all purposes in the United States is supplied by subsurface sources. It is estimated that ground water comprises at least 95 percent of the Nation's fresh-water reserves, with surface water accounting for only 5 percent of the total.[1] Burgeoning requirements for fresh water and limited surface water supplies almost certainly will result in increasing utilization of the ground-water resource to satisfy future water needs.

Although ground waters are less easily polluted than surface waters, the activities of a growing and affluent population are increasingly subjecting subsurface waters in the United States to potential pollution. About 13 million septic systems serving approximately 50 million people are presently in use in the United States,[2] and numerous lagoons or leaching ponds are employed for disposal of domestic, industrial, and agricultural wastes. Soil treatment systems, involving application of high volumes of liquid wastes to large areas of surface soil, as well as water reuse systems entailing percolation of treated waste waters through the earth to recharge ground water, are being utilized with increasing frequency. Sanitary landfills are used for disposal of much of the country's solid waste, and large quantities of chemicals are applied to the soil in intensive agricultural operations. Transport and storage of commercial chemicals, both inorganic and organic, entail accidental introduction of these substances into the ground as a result of transport vehicle accidents or leakage of storage vessels and pipelines. Subsurface disposal of wastes by means of injection wells is employed by the oil industry for disposal of brines, and is becoming increasingly attractive to industries with problem wastes as a means to avoid either excessive waste treatment expenses or violation of surface water quality standards. Also, artificial recharge of ground-water aquifers by direct

injection of surface waters is being practiced to store and conserve surface waters, or to maintain a hydraulic head to prevent contamination of fresh-water aquifers with undesirable waters such as sea water. In total, these activities entail the potential for introduction into subsurface waters of a vast array of pollutants, including a myriad of synthetic organic chemicals. It is imperative that this potential for pollution of ground waters be thoroughly evaluated in order that procedures necessary to prevent or minimize such pollution may be instigated. This need is particularly urgent because pollution effects almost invariably persist much longer and are more difficult to eradicate in ground waters than in surface waters.

Examination of the major potential sources of ground-water pollution reveals that pollutants are most likely to enter ground waters by either of two principal pathways: percolation through the unsaturated portion of the earth's crust above the water table or discharge directly into the subsurface water. Both during percolation through the crust of the earth above the water table and within saturated ground-water zones pollutants are subject to possible sorption, abiotic chemical alteration, and chemical alteration resulting from biological activity. Of the three possibilities, biochemical alteration may well be of greatest potential importance in determining the ultimate effect of a pollutant on ground-water quality.

The topmost layers of the earth's crust, comprising that region considered by the soil scientist as true soil as opposed to subsoil and underlying substratum, have been rather thoroughly studied, principally because of their importance to agriculture, and have been shown to comprise a region of relatively intense biological activity. Hence, pollutants percolating through the soil zone of the earth's crust are known to be subject to potential biochemical alteration as the result of the metabolic activities of a multitudinous population of biochemically diverse microorganisms within this region. However, those regions of the earth's crust lying below the soil zone, including both unsaturated zones above the water table and saturated ground-water zones, have received much less attention in regard to their biological activity than has the soil zone. Consequently, the possibility of biochemical alteration of pollutants in these regions, as well as possible effects of pollutants on subsurface ecosystems, has been given little serious consideration.

With increasing release of pollutants into the crust of the earth through accidents, waste disposal, agricultural activitics, subsurface disposal, and artificial recharge, questions regarding subsurface biological activity and attendant biochemical alteration of pollutants are assuming great importance in regard to control of ground-water pollution. This is especially true since many pollutants are relatively intractable synthetic organic compounds which may survive passage through the upper soil layers and since many pollutants may be introduced directly into ground water without ever passing through the soil zone. Knowledge of the biological activity which occurs or can be expected to occur in the subsurface environment is a necessary component for predicting the probable impact of human activities on the ground-water resource and for planning and conducting such activities to minimize the threat to ground-water quality.

Information pertaining to subsurface biological activity is relatively scarce and widely scattered through the literature of several scientific disciplines, notably geohydrology, geochemistry, geomicrobiology, microbial ecology, and petroleum microbiology. This paper presents a discussion of subsurface microbial activity in relation to ground-water pollution as reflected by available references in these diverse literatures. Principal attention has been devoted to those regions of the earth's crust lying below the soil zone, since numerous compilations of information concerning biological activity in the soil are widely available. The first portion of the paper is devoted to a discussion of the subsurface environment in terms of those factors likely to be of most significance in regard to the development of biological systems. This is followed by a discussion of investigations pertaining to subsurface biological activity, including techniques and problems in studying subsurface biology, results of field investigations of subsurface microbial activity, and the significance of such investigations in regard to ground-water pollution and need for additional studies.

SECTION IV

THE SUBSURFACE AS A BIOLOGICAL HABITAT

The upper continental crust of the earth may be described in a very general way as consisting of crystalline basement rock interspersed with and overlain by sedimentary and volcanic rocks in deposits of varying sizes, shapes, and depths. (The term rock is used here in the broad sense, meaning any solid constituent of the earth's crust.) The basement rock consists mainly of granitic rocks with masses of metamorphosed volcanic and sedimentary rocks, while the sedimentary and volcanic deposits are comprised of unconsolidated rocks such as clay, silt, sand, gravel, and volcanic ash and consolidated rocks such as shale, sandstone, limestone, dolomite, gypsum, anhydrite, rock salt, and lava. These rock materials, together with the liquids and gases filling the voids or interstices within them, comprise the subsurface environment in which subsurface ecological communities must exist and function.

The subsurface environment is a highly structured environment characterized by limited open space. Like the surface environment, it is not homogeneous but varies from point to point, encompassing a wide spectrum of environmental possibilities. In appraising the subsurface as a potential biological habitat, the limitations imposed by these environmental possibilities on biological systems are of principal concern. The nature and extent of these limitations are best revealed by examining available information concerning the subsurface environment in regard to those factors likely to be of most significance in controlling the development of subsurface biota, including spatial limitations, water temperature, hydrostatic pressure, oxidation-reduction conditions, and nutrients.

SPATIAL LIMITATIONS

Organisms which may inhabit the subsurface will be essentially restricted to the space provided by the voids, or interstices, of the rock comprising the upper portion of the crust of the earth. This fact would appear to virtually eliminate subsurface regions below the soil zone as potential habitats for any life forms other than microorganisms. Of principal

concern, therefore, is the "living space" which interstices in the rocks of the subsurface might afford to microorganisms.

The interstices of the rocks which comprise the earth's crust reflect the diverse geological processes by which the rocks were formed and later modified. They are of two major classes: original (or primary) interstices, which came into existence when the rocks were formed; and, secondary interstices, resulting from modification of the rock by processes to which they were subjected after formation. Original interstices consist of spaces between adjacent fragments of sedimentary rocks and cavities or intercrystal spaces formed in igneous rocks during their congealing. Secondary interstices are mainly joints, fractures, and solution cavities. Interstices differ widely in size, shape, arrangement, and aggregate volume. They range in size from large solution cavities through all gradations to minute pores and may be closely interconnected or relatively isolated.

Porosity is a measure of the interstitial space of a rock and is usually expressed as the percentage of the total volume of that rock which is occupied by interstices. The ability of a rock to transmit fluid, called its permeability, provides an indication of the size and degree of interconnection of the interstices. In ground-water investigations, permeabilities are usually stated in terms of the coefficient of permeability, which is defined as that quantity of water in gallons per day which will flow through a cross-sectional area of one square foot of rock under a hydraulic gradient of one foot per foot.

In Table 1 are presented some representative porosity and permeability ranges for selected sedimentary rocks, as given by Walton[3] and Todd.[4] As these data show, the aggregate volume of the interstices in sedimentary rocks usually comprises a significant portion of the total volume of these rocks. However, the individual interstices may sometimes be extremely small or poorly interconnected, resulting in very low permeability of the rock even though its porosity is appreciable, as in the case of clay, silt, shale, and some sandstones. Crystalline rocks are usually quite low in both porosity and permeability, unless they contain significant secondary interstices.

Table 1. REPRESENTATIVE POROSITY AND PERMEABILITY RANGES FOR SELECTED SEDIMENTARY ROCKS

Rocks	Porosity, %	Permeability, gpd/ft^2
Clay	45-55	0.001-2
Silt	40-50	0.001-2
Sand	35-40	100-3000
Gravel	30-40	1000-15000
Sandstone	10-20	0.1-50
Shale	1-10	0.00001-0.1
Limestone	1-10	No data available

Porosity data indicate that the total interstitial space per unit volume of most rocks comprising the upper crust of the earth, with the possible exception of massive crystalline rocks containing no joints or fractures, is adequate to permit at least some microbial activity, provided the sizes of the individual interstices are sufficient to accomodate the dimensions of the microorganisms. Permeability, however, provides only a qualitative indication of interstice size, and the sizes of interstices in most rocks have not been quantitatively defined. Hence, it cannot be presently ascertained if spatial limitations definitely preclude microbial activity in any subsurface regions above those extreme depths where interstitial space becomes practically nonexistent. However, Berea sandstone, a rather dense rock with permeability in the neighborhood of 1 gpd/ft^2, was reported by Kalish, et al., to have a mean pore diameter of 7 to 12 microns,[5] which would accomodate the dimensions of most bacteria, particularly the smaller organisms; and bacteria (^{32}P-labeled-Serratia marcescens) were found by Myers and McCready to be transported by water through a 2-inch by 14.25-inch core of this rock in 84 hours.[6] The latter investigators also reported that S. marcescens passed through Mississippian and Early Devonian limestone and Late Mesozoic sandstone with permeabilities in the proximity of 0.1-0.001 gpd/ft^2. These data suggest that bacteria might find adequate space to exist and function in wide regions of the subsurface. Certainly, spatial

limitations would not appear likely to exclude as microbial habitats those subsurface regions of sufficient porosity and permeability to permit transmission and storage of appreciable quantities of water. These are, of course, the regions of greatest interest in regard to ground-water pollution control.

Spatial limitations of the subsurface environment are also of concern in the transport of microorganisms into and within subsurface regions by moving water. Obviously, physical difficulty in negotiating small interstices as well as possible sorption on solid surfaces are likely to greatly impede the movement of microbes through the interstitial space of subsurface rocks. However, as previously noted, investigations by Myers and McCready indicated that the bacterium S. marcescens can, with time, pass through sandstones and limestones of low permeability.[6] Also, several investigations, reviewed by Mallman and Mack,[7] revealed considerable potential for transport of bacteria through relatively permeable sands by moving water. These studies, which pertained to the pollution of ground water by microbes from sewage, actually indicated that coliform organisms from sewage did not move great distances through the sands studied. However, the principal deterrent to movement of the coliforms was apparently not physical impediment within the interstices of the sand, but rather the development of a mat of solids from the sewage at the interface where the liquid entered the sand with resulting removal of the organisms from the infiltrating water by filtration or sorption on this mat. Before development of the mat, coliform organisms moved rather rapidly through sand for distances as great as 30 m (100 ft). It seems likely that this distance was largely a function of the life span of the coliforms in an environment which was not most conducive to their growth, rather than an indication of physical impediment of movement of the microbes.

These investigations suggest that bacteria which are capable of adapting to the subsurface environment might be able to gain access even to deep subsurface habitats by moving, perhaps over long periods of time, from the surface with percolating water through the interstices of the rocks comprising the earth's crust.

WATER

Regardless of the nature of their habitat, microbes are fundamentally aquatic organisms which require free water for growth. The cells must be surrounded by a layer of water that is adequate to permit diffusion of nutrients and toxic products and to maintain the internal water budget in order that metabolism and reproduction may proceed. It is necessary, therefore, in considering the subsurface as a possible microbial habitat, to examine the distribution and nature of subsurface water.

Subsurface water is generally distributed vertically within the earth's crust in two major zones, the zone of aeration (also called the zone of infiltration or vadose zone) and the zone of saturation. The zone of aeration, in which the interstices of the rocks comprising the crust of the earth contain varying proportions of water and gas, overlies the zone of saturation, in which the interstices are completely filled with liquid, usually water. The depth to the upper boundary of the zone of saturation at a specific geographical location depends on geological and climatic factors and may vary from essentially the surface to many hundreds of feet. Since the zone of aeration lies between the land surface and the zone of saturation, its thickness varies with and is equivalent to the depth to the zone of saturation.

The zone of aeration may be divided into three subzones: the soil water zone; the intermediate zone; and the capillary zone.

The soil water zone extends from the surface down through the major root zone and varies in depth with soil type and vegetation. Water contained within this zone may be classified as follows:

1. Hygroscopic water - water which is very strongly adsorbed by soil particles, forming a thin film around them; it is present even in extremely dry soils in times of drought and in deserts, but is generally unavailable to plants and soil microbes.

2. Capillary water - water which is held in the soil interstices by surface tension, and which is available to soil organisms.

3. Gravitational water - excess water which is not held by surface tension or adsorptive forces, and which drains into underlying zones under the influence of gravity; it is normally present only during periods of relatively high infiltration resulting from rainfall, irrigation, or liquid waste disposal to the soil.

The intermediate zone extends from the lower edge of the soil water zone to the top of the capillary zone. It is primarily a region through which water must pass to reach the zone of saturation and may be nonexistent or several hundred feet thick. It contains gravitational water during periods of infiltration and otherwise holds a significant portion of capillary water, sometimes called pellicular water.

The capillary zone extends from the top of the zone of saturation to the limit of capillary rise of water. Its thickness is a function of the size of the interstices of the rock in which it occurs. Hence, it may extend several feet in clay and only a fraction of an inch in gravel.

The zone of saturation may extend to great depths within the crust of the earth, particularly in deep sedimentary basins. Kuznetsov, et al., point out that Filatov has estimated gravitational water to be capable under natural conditions of moving through sedimentary rock to the depth of about 4,000 m (13,000 ft).[8] Some wells have actually obtained water at depths of more than 3,000 m (10,000 ft).[9] Water contained in the zone of saturation is by definition ground water. The vertical distribution of ground water within the zone of saturation is not usually uniform but reflects the varying porosity and permeability of the successive strata of rocks comprising the earth's crust at any given geographical location. Hence, there may be at successively greater depths several saturated strata of sufficient porosity and permeability to yield significant quantities of water, separated by other strata which are, in the vast majority of cases, also saturated but which are relatively impermeable. Of course, regions in which water has been displaced by petroleum liquids or gases are often found in the zone of saturation, usually at greater depths. These regions are, in a sense, anomalous.

Movement of subsurface water within the zone of aeration is essentially vertical in direction, while the movement of ground water in the zone of saturation is mainly lateral, towards points of discharge to the surface. The rate of movement of ground water is quite slow, usually being measured in feet per day or feet per year. In the permeable formations comprising the upper part of the zone of saturation rates of movement are usually highest, so that residence times for water in these formations most generally do not exceed a few years. Water movement in the deeper permeable strata of the zone of saturation, ranging in depth from hundreds to thousands of feet, is generally much slower, with residence times of decades to thousands of years being common. Some waters deep within the zone of saturation, usually several thousand feet below the land surface, are essentially stagnant, exhibiting no discernable movement. These waters appear to have been trapped for geologic ages in permeable formations which are completely isolated from the natural circulating system of the hydrologic cycle by impermeable strata.

Because of the great solvent power of water, ground water usually reflects to some extent the chemical composition of the rock formations through which it moves. In general, the mineral content of ground waters increases with depth and may reach levels in excess of 20 percent total mineral content in deep formations, particularly those which appear to be hydrogeologically isolated.[8,10] Ground waters of high mineral content are also found in relatively shallow rock formations which are partly composed of salt beds deposited from ancient seas.

This description of the nature and distribution of subsurface water, though brief and oversimplified, shows clearly that water is virtually ubiquitous in the subsurface environment but varies considerably in quantitative distribution, movement, and mineral content.

The lowest relative humidity at which microbial growth is possible depends on the species,[11] but most soil microbes cannot grow when the soil contains only hygroscopic water. Hence, microbial growth in the soil water zone may be greatly retarded during periods of drought, but quickly resumes, often at increased levels, when water replenishment occurs.[12] In the deeper regions of the zone of aeration, however, it seems unlikely that moisture levels would ever become so low as to effectively limit microbial activity.

In those regions of the zone of saturation where ground waters are highly mineralized the availability of water to many microbes would, in effect, be severely restricted because of high osmotic pressures. However, bacteria differ greatly in their ability to withstand and adapt to high osmotic pressure, and many species have been found to grow in highly mineralized waters, including some which tolerate as much as 30 percent salt.[13] Therefore, considering the general abundance of water in the zone of saturation, it seems a logical conclusion that practically no subsurface environmental possibility would be completely hostile to all microorganisms because of limited availability of water.

TEMPERATURE

Temperature is one of the most important environmental factors controlling the metabolic activity of microorganisms in any type of habitat, and considerably information has been developed concerning the relationship of temperature to the growth and survival of microorganisms. In general, microbial metabolic activity is roughly doubled for each 10°C rise in temperature until a temperature is reached at which the rate of thermal denaturation of enzyme proteins exceeds the rate of thermal stimulation of enzyme reaction rates. For mesophilic organisms, which comprise by far the greatest number of microbes, denaturation becomes significant above 40°C; hence, the optimum growth range for these organisms is 25-40°C. Thermophilic organisms have an optimum growth temperature of 55-60°C, but some can continue to grow at temperatures as high as 75-90°C at atmospheric pressure, evidently because they produce enzymes which are less prone to heat denaturation. The maximum temperature that all but a few heat-resistant spore-forming bacteria can tolerate without being killed is normally considered to be 100°C, but most microorganisms are actually killed by temperature much above 50°C.[14]

The minimum temperature limits at which all microorganisms cease to grow in nature are determined primarily by their need for water in the aqueous phase and may be as low as -15°C in waters with a high concentration of solutes.[15] Many psychrophilic bacteria are found in nature which can grow fairly rapidly between 0° and 5°C. However, the optimum growth temperatures for many psychrophiles actually are often in the same range of 25°-40°C as most other bacteria.[14,16]

The temperature tolerances of microorganisms may be affected by other environmental factors. For example, high acidity seems to lower the maximum temperature at which growth may occur.[17] Pressure and temperature are definitely interrelated, the maximum temperature for growth being raised 5°-20°C by compression according to ZoBell.[18]

Temperatures in the earth's crust rarely fluctuate, except in the topmost layer of approximately 10 m (33 ft) which may be affected by seasonal temperature variations.[8] The normal ground-water temperature at depths of 9-18 m (30-60 ft) roughly corresponds to the mean annual air temperature of the given region and ranges from about 3°C (37°F) to 25°C (77°F) in the United States.[1] As the depth increases, the temperature of the subsurface rocks and waters increases; this increase is roughly estimated to be 30°C per 1,000 m (3,281 ft).[19]

Based on upper temperature limits of 80°C and 100°C, respectively, for microbial growth and survival, and assuming a 30°C increase in temperature per 1,000 m of depth, it would appear that high temperatures would likely prevent most microbial activity in subsurface environments lying below approximately 1,800 m (5,900 ft) and would virtually preclude the existence of microbes in those environments at depths below about 2,500 m (8,200 ft). These values are, however, only very rough guidelines subject to wide variation, since temperatures within the earth's crust at any particular geographical location are likely to reflect not only depth but also geologic structure and geothermal activity. Hence, high temperatures may sometimes occur at relatively shallow depths, while temperatures considerably lower than predicted may be encountered in deep formations. For example, on the basis of 4,500 thermographs and 700 individual measurements from more than 5,000 deep bore holes throughout the European USSR, Pokrovskii, et al., concluded that the 100° isotherm in this region lay at varied depths generally ranging from 2,900 to 5,500 m (9,500 to 18,100 ft).[20] In a few areas, however, temperatures of 100°C were encountered within 300-400 m (1,000-1,300 ft) of the surface because of thermal springs, and in other areas the 100° isotherm was indicated to be as deep as 10,000-15,000 m (32,800-41,200 ft) because of low geothermal activity.

It is obvious, therefore, that temperature extremes sufficient to preclude microbial activity are not likely to occur in those regions of the subsurface that are of greatest concern in regard to ground-water pollution, with the possible exception of a few regions of extreme geothermal

activity and some very deep strata which may be employed for subsurface disposal of waste waters. In fact, the elevated constant temperatures of 25-40°C which are likely to occur in many subsurface regions would be ideal for growth of mesophilic organisms.

HYDROSTATIC PRESSURE

The pressure within the crust of the earth increases progressively with depth. This increase is due principally to the weight of the overlying rocks and of the water contained within the rock interstices. The hydrostatic pressure within rock interstices, in which any subsurface microbial activity must occur, usually is not influenced to any great extent by the overburden pressure exhibited by the rocks but approximates the pressure which would be produced by a column of water extending from the surface to the depth of the measurement. Hence, interstitial hydrostatic pressure generally increases downward at an average rate of 0.1 atm per meter or about 0.43-0.47 psi per ft, although pressures considerably higher or lower than predicted may be encountered with some frequency.[21] For example, in an extensive study of pressures in 76 oil wells in the Gulf Coast Region of Texas and Louisiana actual pressures were found to agree quite well with predicted values down to a depth of about 2,200 m (7,200 ft), ranging from 37 atm at about 365 m to approximately 221 atm at 2,160 m.[22] At greater depths positive pressure deviations were quite common, with a pressure of 530 atm at about 3,200 m (10,200 ft)--a deviation of 64 percent--being noted in one case. These excessive pressures may have been due to the development of gases and to the partial transmission of overburden pressures to confined interstitial fluids in isolated deep formations.

Kuznetsov,et al., concluded, on the basis of pressures observed in oil wells in the USSR, that the hydrostatic pressure should not exceed 300-400 atm in the majority of subsurface regions where temperatures are not too high for microbial activity.[8] This appears a valid assumption, although there may well be exceptions in regions of low geothermal activity.

Microorganisms differ considerably in their response to hydrostatic pressure. Some may survive for prolonged periods at pressures greater than 2,000 atm, but none have been found that can grow at such high pressures. Most microbes which normally are found growing in habitats near atmospheric pressure appear

capable of at least some growth at 200-300 atm at 30°C, although some species are completely inhibited or killed by these or even lower hydrostatic pressures.[16,23,24] At higher pressures the growth of organisms from surface or near-surface habitats is usually severely to completely retarded, and the survival of such microbes for significant periods at pressures of 500-600 atm is doubtful.

On the other hand, many microorganisms isolated from deep sea habitats have exhibited the ability to grow readily at pressures above 400 atm during laboratory studies. Sulfate-reducing bacteria from the Philippine Trench (depth about 10,000 m) were observed by ZoBell to grow very slowly at pressures as high as 1,400 atm, but relatively few species of microbes from all habitats have been successfully cultivated under laboratory conditions at pressures in excess of 800 atm.[25]

Pressure-tolerant microbes which have been investigated exhibit significant variability in regard to the range of pressures within which they are capable of growth. Many also can grow at low hydrostatic pressures. This is illustrated by an investigation of ZoBell and Budge, who found that four of eight species of marine facultative anaerobes capable of growing at 1,000 atm grew best at 1 atm, while the optimum pressures for growth of the other four species were in the range of 100-200 atm.[26] Other pressure-tolerant microbes, however, grow poorly or not at all at low pressures. For example, sulfate-reducing bacteria from the Weber Deep in the Indian Ocean were cultivated for several years at 700 atm at temperatures of 3-5°C by ZoBell and Morita but failed to grow at 1 atm.[27]

Microorganisms growing at pressures near their maximum tolerance levels often exhibit indications of altered metabolic processes. Filamentous growth resulting from failure of cells to divide in the normal manner is commonly noted, and decreased synthesis of macromolecules such as nucleotides and proteins has been observed by several investigators.[28,29,30] Also, the ability of microbes to grow at high pressures appears to be enhanced by the plentiful availability of organic metabolites (amino acids, vitamins, etc.) in the surrounding medium, which probably reflects decreased synthetic capability at higher pressures.[25] The mechanisms by which increased pressures induce alterations in microbial metabolic processes have not been elucidated, but

inhibition of enzyme systems, possibly through adverse effects on formation of enzyme-substrate complexes, may be involved. Such a mechanism appears plausible because the formation of an enzyme-substrate complex entails an increase in molecular volume, and increased hydrostatic pressure tends to inhibit reactions requiring increases in molecular volume.[31,32] This mechanism would be consistent with the observation that pressure tolerance levels of microbes are usually raised by limited increases in temperature,[18] since increased temperature tends to increase molecular volume and hence might be expected to attenuate the effects of increased pressure.

In summarizing the information presented above, it seems a reasonable conclusion that hydrostatic pressures are not likely to exclude microbial activity in subsurface regions which are otherwise suitable as microbial habitats. However, pressures in deep formations employed for subsurface disposal of liquid wastes may be sufficiently high to kill or to severely affect the metabolic activity of microbes from surface habitats which may be indigenous to the injected wastes.

OXIDATION-REDUCTION CONDITIONS

Determinations of both molecular oxygen concentrations and oxidation-reduction potentials in subsurface environments are subject to formidable methodological problems because of the difficult accessibility of these regions. Consequently, definitive data concerning subsurface oxidation-reduction conditions are scarce. However, those data which have been reported, coupled with general observations concerning the probable availability and consumption (reduction) of molecular oxygen and other reducible substances in subsurface regions, provide a limited insight regarding probable oxidation-reduction conditions in subsurface zones.

In the zone of aeration, gas in the rock interstices is for the most part in direct communication with the atmosphere and, hence, is potentially subject to constant interchange with atmospheric gases. Interchange results from molecular diffusion of gases through the rock interstices and from actual displacement of interstitial gas due to diurnal heating and cooling of the earth's surface, changes in barometric pressure, and intermittent filling of interstices by gravitational water with subsequent displacement of this water by

fresh atmospheric gas. It has been widely assumed, therefore, that high oxygen tensions generally prevail within the zone of aeration. However, the situation is probably not so simple as this assumption implies.

Regions of oxygen deficiency are known to occur with some frequency in the soil water subzone of the zone of aeration, even though oxygen replenishment by gas interchange should be most effective in this region by virtue of its close proximity to the atmosphere. These oxygen-deficient regions probably occur because of an unfavorable balance between oxygen replenishment by gas interchange, which appears to be restricted by interstitial spatial limitations, and oxygen utilization by soil microorganisms. For example, it is known that soils which are rich in organic matter are likely to become deficient in oxygen even near the soil surface;[33] also, soils of small particle size, and hence low permeability, have been found to exhibit oxygen deficiency at shallow depths, while coarser soils of greater permeability maintained a high oxygen tension under similar conditions.[34] In addition, anaerobic microenvironments are believed to occur commonly in the soil water zone, even in well-aerated soils.[11] These microenvironments are thought to consist of soil aggregates which have developed oxygen-deficient regions within their interiors because of inadequate diffusion of oxygen through water surrounding and within the aggregates and microbial depletion of oxygen on their peripheries.[35]

Microbial activity and, hence, oxygen consumption is almost certainly significantly less in the intermediate and capillary subzones of the zone of aeration than in the soil subzone in most situations. However, the rate of gas interchange with the atmosphere must surely be related to depth and to permeability of the rock comprising the zone of aeration and is therefore likely to be low in these deeper regions, particularly in formations of low permeability. Also, the probability of survival of molecular oxygen during movement through the rock interstices towards the deeper regions of the zone of aeration could be relatively low if microbial activity in the soil zone were high. It would appear, therefore, that regions of oxygen deficiency may be at least as likely to occur in the deeper subzones of the zone of aeration as in the soil water subzone.

In summary, it appears logical to suspect that the oxidation-reduction conditions at any specific point in the zone of aeration will reflect the geology of the zone of aeration, the

depth of this point within the zone of aeration, and the microbial activity in the vicinity of the point and in the overlying strata and, hence, may well vary over a wide range of possibilities.

Oxidation-reduction conditions in the zone of saturation likely will reflect the balance between: (1) the rates of processes which result in the reduction of molecular oxygen and other reducible substances in ground water; and, (2) the rates of entry into ground water of recharge water containing oxygen and other reducible substances and of diffusion into ground water of oxygen from the zone of aeration. Hence, redox conditions at any specific point in the zone of saturation will likely depend on the several factors presented below.

1. Redox conditions in the overlying zone of aeration. -- Obviously, the zone of saturation can receive little or no oxygen replenishment from the zone of aeration if the gas at the interface between the two zones is deficient in oxygen.

2. Distance from the interface between the zones of aeration and saturation. -- Replenishment of oxygen and other electron acceptors becomes more difficult as the distance from the air-water interface in a geological formation increases, since diffusion of oxygen through water and vertical mixing of ground water are usually very slow processes.

3. Extent of microbial activity in the ground water. -- Although abiotic reductive processes may be of some importance in specified ground waters, particularly in regions of high temperature and pressure, those processes which are most likely to result in reduction of molecular oxygen and other reducible substances in ground water are probably associated with microbial activity. Hence, the ultimate level of reduction which is attained in a particular region of the zone of saturation will likely depend to a large extent on the level of microbial activity in that region.

4. Rate of movement of ground water. -- The longer the residence time for ground water in a specific subsurface region, the longer it will be subject to any reductive processes which may be operating there; hence, a reduced environment could develop in a saturated subsurface

region where water movement is very slow, even though the rates of processes resulting in reduction of molecular oxygen and other reducible substances may also be slow in this region.

5. Rate of ground-water recharge. -- Recharge of ground water ordinarily results in replenishment of oxygen and other reducible substances in the zone of saturation by virtue of the dissolved oxygen and other easily reducible substances such as ferric iron, nitrate, sulfate, and certain organic compounds which may be present in the recharge water. Rate of ground-water movement also is usually related to rate of recharge.

Since the deeper regions of the zone of saturation are far removed from the zone of aeration and the rates of ground-water movement within these regions and the rates at which they are recharged range from very low to essentially zero, it appears unlikely that appreciable oxygen and other easily reduced substances could be present in them. This supposition is supported by data obtained in the USSR during studies related to the genesis of and exploration for petroleum. For example, Kuznetsova found waters in deep formations in the oil fields of the Kuybyshev region to have redox potentials (E_h) ranging from +20 to -250 mv.[36] Also, Al'tovskii detected no oxygen in water from 18 wells in the Groznyy oil regions ranging in depth from 225 to 2,097 m (approximately 740 to 6,900 ft).[37] The average redox potential, expressed as rH_2, for these waters was 11.0 ($rH_2 = E_h/0.029 + 2pH$; hence at pH = 7.0, rH_2 = 11.0 would be equivalent to E_h = -87 mv). There is little doubt,therefore, that the deeper regions of the zone of saturation generally comprise a reducing environment.

On the other hand, considerable variations in rates of reductive processes and replenishment of oxidized substances appear possible in the upper regions of the zone of saturation. Hence, it seems likely that oxidation-reduction conditions in these regions may vary over a significant range of possibilities, although somewhat reducing conditions would appear to be generally favored. Data obtained by Al'tovskii from two wells tapping an artesian aquifer in the USSR are of interest in regard to this hypothesis.[37] One well which was in the recharge area of the aquifer was approximately 75 m (245 ft) deep and produced water with an rH_2 of 27.2 (E_h = 382 mv at pH 7) and an oxygen content of 4.8 ppm. The second well,

which was in the discharge region of the aquifer, was about 20 m (65 ft) deep and produced water with an rH_2 of 14.3 (E_h = 10 mv at pH 7) and which contained no oxygen. The presence of oxygen and a high redox potential in water from the deeper well apparently reflected the replenishment of oxygen in the zone of saturation by oxygenated recharge water and also possibly by diffusion from the nearby zone of aeration. The water in the second well was separated from the immediate overlying zone of aeration by a relatively impermeable formation and from the recharge region by a long distance. Hence, there was little chance for replenishment of the oxygen which was consumed during its movement through the aquifer.

The observation of redox potentials ranging from +540 mv to -380 mv in water samples obtained just below a water table ranging from 10 to 70 ft (3-21 m) beneath the surface in the valley of the South Platte River of Colorado may provide a further indication of the range of oxidation-reduction conditions which can be expected in the upper regions of the zone of aeration.[38]

The oxidation-reduction conditions in which microorganisms can grow have been cited by Vallentyne as ranging from an upper E_h limit of +850 mv at pH 3 for iron bacteria to a lower limit of -450 mv at pH 9.5 for sulfate bacteria.[15] The total exclusion of microbial activity in subsurface environments by unfavorable oxidation-reduction conditions therefore seems unlikely, although the possibility exists that some deep regions of the zone of saturation might become so reduced as to preclude microbial growth. However, since different species of microorganisms usually are able to grow well only within a relatively narrow range of redox values, the oxidation-reduction conditions that prevail within a subsurface region will limit the species of microbes which may successfully inhabit that region and will often control the metabolic pathways and products of these microbes. For example, sulfate-reducing bacteria require an environmental E_h of -200 mv or less for initiation of growth,[39] and reduction of nitrate by facultative microbes in soil has been found not to proceed unless the redox potential is less than +338 mv at pH 5.1.[40] Also, metabolic activity in soils apparently changes from aerobic to anaerobic, with concomitant accumulation of partially oxidized organic compounds, when the concentration of oxygen declines below 3×10^{-6} m.[41] The implications of oxidation-reduction conditions in regard to the fate of pollutants in subsurface waters are obvious.

NUTRIENTS

A microbial habitat must provide to resident microorganisms the nutrients which they require for synthesis of protoplasmic constituents and for generation of energy needed for conduct of life processes. For synthesis of protoplasm a carbon source, either carbon dioxide or organic matter, must be available, along with smaller quantities of nitrogen, phosphorus, and sulfur in either organic or inorganic form, and low levels of various minerals. Generation of energy by chemotrophic organisms (the absence of solar radiation obviously excludes phototrophs from subsurface environments) requires the availability of: (1) suitable electron donors, such as oxidizable organic compounds, or, for growth of chemolithotrophs, oxidizable inorganic substances such as molecular hydrogen, reduced sulfur compounds, ammonia, nitrite, and ferrous iron; and, (2) suitable electron acceptors including molecular oxygen, nitrate, sulfate, carbon dioxide, and simple organic compounds.

Potential sources of carbon for microbial utilization are relatively plentiful in many subsurface environments, since the upper continental crust of the earth is variously estimated to contain in the neighborhood of 10^{19}-10^{20} Kg of carbon.[42,43] Most of this carbon is present as inorganic compounds, principally carbonates, but an appreciable portion occurs as organic matter, much of which was incorporated into sedimentary deposits at the time of their formation and has not been completely mineralized over geological ages. Large quantities of organic compounds are found concentrated in petroleum deposits but vastly greater amounts are dispersed throughout sedimentary rocks in a finely disseminated state. For example, analysis of 35,000 samples of ancient sediments obtained from sedimentary formations in the United States revealed these materials to contain an average of 1.5 percent organic matter.[44] Similar study of 2,000 samples of recent sediments, mostly of marine origin, showed organic matter ranging from 0.5-10 percent.[45] Other geochemical studies have generally confirmed the apparently ubiquitous occurrence of significant quantities of organic matter in sedimentary deposits, although recent terrigenous sediments have received relatively little investigation in this regard.

Organic matter in sedimentary rocks may be grossly classified as bitumen, comprising organic substances which are extracted by neutral organic solvents, and kerogen, consisting of organic materials which are not readily soluble in such solvents. The

bitumen fraction usually contains numerous hydrocarbon materials, fatty acids, porphyrins and many other substances; its composition is likely dependent to some extent on the organic solvent employed for extraction. The kerogen fraction constitutes the bulk of the organic matter in subsurface environments, usually comprising 90 percent or more of the total organic content of sedimentary materials.[46] The composition of this fraction is very complex and subject to considerable variation; but, in general, it appears to consist in large part of humus-like materials.

Available information also indicates that essentially all subsurface waters probably contain significant quantities of dissolved organic matter, as illustrated by Table 2. This table presents representative data for organic carbon content for both fresh and highly mineralized ground waters obtained at depths ranging from 6 m (19.7 ft) to 3,049 m (10,003 ft) at a number of different geographical locations and, hence, provides some indication of the levels of dissolved organic matter occurring in subsurface waters. This organic matter probably results both from leaching of organics from sedimentary rocks into ground water and transport of fresh organic matter from the surface by recharge water. Humic substances, naphthenic acids, fatty acids, and phenols are known to comprise part of the dissolved organic matter in ground waters near petroleum deposits, but the specific composition of the naturally occurring organic matter of ground waters generally remains largely unknown.

The growth of heterotrophic (chemoorganotrophic) bacteria may often proceed when very low levels of organic matter are available, ZoBell, et al., having observed marine bacteria growing readily at concentrations of organic substances lower than 0.1 mg/l.[49,50] It would appear likely, therefore, that the organic substances present in most subsurface environments of sedimentary origin are sufficient in quantity to support microbial activity, both as sources of carbon for synthesis of protoplasm and as electron donors for generation of energy. However, microbial activity in the subsurface may be limited by the quality, rather than the quantity, of subsurface organic matter. For example, much of this material may, as noted above, consist of substances resembling soil humus components which yield to microbial degradative processes only very slowly, even under the most favorable conditions. Also, some compounds may not be utilized by microbes in the absence of oxygen per se,[51] and some may even be microbial inhibitors.

Table 2. CONTENT OF ORGANIC CARBON IN REPRESENTATIVE GROUND WATERS

Location	Depth	Organic Carbon, mg/l	Reference
Moscow Basin (USSR)	6 m (19.7 ft)	0.3-0.6	Al'tovskii, et al., 1961[37]
Moscow Basin (USSR)	80-260 m (262-853 ft)	0.64	Al'tovskii, et al., 1961[37]
South Platte River, Valley, Colorado	6.4-10.3 m (21-34 ft)	7-51	Stewart, et al., 1967[38]
Western Turkmenia (USSR)	512 m (1,679 ft)	4.2	Al'tovskii, et al., 1961[37]
Ob'irtysh Basin (USSR)	664-672 m (2,178-2,205 ft)	1.23	Bars and Nosova, 1964[47]
Ob'irtysh Basin (USSR)	2,314-2,317 m (7,559-7,602 ft)	5.28	Bars and Nosova, 1964[47]
Orenburg Region (USSR)	448-452 m (1,470-1,483 ft)	5.0	Bars and Kogan, 1964[48]
Orenburg Region (USSR)	1,633-1,639 m (5,358-5,377 ft)	1.77	Bars and Kogan, 1964[48]
Orenburg Region (USSR)	1,735-1,741 m (5,692-5,712 ft)	28.4	Bars and Nosova, 1964[47]
Kuibyshev Region (USSR)	854-860 m (2,802-2,821 ft)	1.45	Bars and Kogan, 1964[48]
Kuibyshev Region (USSR)	1,360-1,390 m (4,462-4,560 ft)	1.92	Bars and Kogan, 1964[48]
Kuibyshev Region (USSR)	3,036-3,049 m (9,961-10,003 ft)	1.35	Bars and Kogan, 1964[48]

Carbon dioxide is potentially available as a carbon source for chemolithotrophic (autotrophic) microorganisms in many subsurface regions. Inorganic electron donors which might be oxidized by such microbes for generation of energy are also encountered with some frequency in subsurface environments. For example, molecular hydrogen is often present in subsurface gases,[8] and elemental sulfur and hydrogen sulfide are not uncommon in the earth's crust.

Nitrogen, phosphorus, and sulfur are probably present in essentially all sedimentary formations as constituents of organic and/or mineral matter, albeit the concentration of one or more of these elements, most likely nitrogen and phosphorus, may be extremely low in specific locations. However, since the amount of carbon in microbial protoplasm exceeds the quantity of nitrogen, phosphorus, and sulfur, respectively, by approximately five-, twenty-, and one hundredfold, and the latter elements are likely to be recycled in-place in subsurface environments, the levels of these elements required to maintain a minimum level of microbial activity in such environments would appear very low. Hence, it seems unlikely that nitrogen, phosphorus, and sulfur concentrations would often be so low in subsurface environments as to completely preclude all microbial activity, although the probability appears high that limited availability of one or more of these elements in a readily utilized form will restrict the level of microbial activity which can possibly occur in many subsurface regions.

Inhibition of microbial activity in subsurface environments solely because of unavailability of mineral elements would also likely be exceptional, since all subsurface waters are mineralized to some extent due to leaching of the rocks with which they come in contact, and the concentrations of inorganic ions required for microbial growth are usually extremely low. In this connection, Kartsev states that the presence of mineral compounds necessary for growth of hydrocarbon-utilizing microbes has been established in a wide variety of subsurface formations, although clay interbeds deficient in the minerals required for development of these bacteria have been observed in rare cases.[52]

As noted in the preceding discussion of subsurface redox conditions, molecular oxygen, the electron acceptor required by obligate aerobes and usually preferred by facultative microbes, is undoubtedly absent from the deeper regions of the zone of saturation and is likely available in variable

and often limited quantities in the upper regions of the zone of saturation and in the zone of aeration. However, other substances which serve as terminal electron acceptors for anaerobic organisms or which may be utilized as alternate electron acceptors by facultative organisms are widely distributed in subsurface regions. Sulfate, the required terminal electron acceptor of the obligately anaerobic sulfate-reducing bacteria, is probably present to some extent in most subsurface waters, often in relatively high quantity. Carbon dioxide, as previously noted, is present in many subsurface environments and may be utilized as terminal electron acceptor by several species of methane bacteria. Nitrate, the alternate electron acceptor for many facultative microbes, occurs often in subsurface waters, particularly at relatively shallow depths. Simple organic compounds which may serve a number of microbes as electron acceptors via fermentative metabolic pathways are also likely present in many subsurface environments, although such substances probably become progressively more scarce with increasing depth.[53] In total, the availability of electron acceptors is probably sufficient in many subsurface regions to support at least a minimum level of microbial activity. However, the rates at which electron acceptors can be replenished in various subsurface environments appear likely to limit the levels of microbial proliferation which may be sustained therein. Such replenishment rates are undoubtedly very low in many subsurface regions, particularly in deeper strata of the zone of saturation. Insufficient availability of suitable electron acceptors to sustain significant microbial activity may well be a principal factor in the survival for geological ages of organic matter, particularly petroleum deposits, in the earth's crust.

GENERAL ENVIRONMENTAL OBSERVATIONS

Environmental factors other than those discussed above may also affect microorganisms in subsurface regions but do not seem likely to preclude microbial activity in such regions except in rare cases. For example, pH may limit the types of organisms which find various subsurface regions to be suitable habitats, and large solid surface areas characteristic of the highly structured subsurface environment may influence microbial activity through sorptive effects on extracellular enzymes, nutrients, and the microbes themselves.

It should also be noted that the various environmental factors, while discussed individually above, are interacting, as illustrated by the interrelation between temperature and pressure noted previously. Definitive experimental data concerning subsurface environmental factors are relatively scarce and incomplete and are not sufficient to define completely the total potential effect of these interacting factors on subsurface microbial activity. Nevertheless, available evidence indicates strongly that the upper continental crust of the earth is generally not a hostile environment for microorganisms, and probably encompasses many regions, particularly those of sedimentary origin, which are quite suitable habitats for many microbial species. The discovery of diverse microbial populations in a number of subsurface regions, as described in the next section of this paper, lends credence to this hypothesis.

The observations presented in this section concern mostly subsurface environmental factors in natural environments unaffected by human activities. Unquestionably such activities could profoundly alter the environmental conditions in many subsurface regions. For example, low levels of readily utilizable carbon sources, electron acceptors, and possibly phosphorus and nitrogen appear most likely to limit microbial activity in many subsurface environments. Disposal or ground-water recharge activities which result in the entry of liquid or solid wastes into regions below the soil zone would be likely to considerably alter this situation in the surrounding subsurface environment.

SECTION V

INVESTIGATIONS PERTAINING TO SUBSURFACE BIOLOGICAL ACTIVITY

Subsurface regions of the earth's crust have never been studied extensively by microbiologists. Early investigations by soil microbiologists on the distribution of microbes at different soil depths indicated the presence of relatively large populations near the surface with numbers decreasing with depth and attaining extremely low levels at the lower boundaries of the soil zone.[54,55,56] From these studies, many microbiologists formed the assumption that biological life below the upper soil zone was either nonexistent or extremely limited. This assumption, when coupled with the immense technical problems and expense involved in subsurface investigations, resulted in many subsequent workers ignoring the subsurface as an area of investigation. Limited numbers of scientists associated with petroleum technology, however, have had a continuing interest in the microbiology of subsurface regions since the sterility of petroleum deposits came under serious question in the 1920s. Their investigations concerning the origin of oil and the development of bacterial methods to aid in the exploration and production of oil are primarily responsible for currently available knowledge concerning microbial life in subsurface regions. Such studies have proved beyond a reasonable doubt that many subsurface regions are not hostile to microbial life and, in fact, contain a varied and apparently active microflora.

Investigations of microbial activity in subsurface regions are discussed below. The techniques and somewhat unique technical problems of the study of subsurface biology are first described; this is followed by a discussion of results obtained in field investigations of subsurface microbial activity; finally, observations regarding the significance of previous microbial studies and the need for additional investigations are presented.

TECHNIQUES AND PROBLEMS IN STUDYING SUBSURFACE BIOLOGY

Obviously, terrestrial environments far below the earth's surface cannot be directly examined because of their inaccessability. Instead, biological activity in subsurface environment must be characterized by examining natural spring waters or by drilling wells and examining the resultant cores and well waters. Spring waters give an indication of the chemical nature of the producing aquifer, but are not necessarily biologically or chemically representative of deep stratal waters.[46,57] In essence, they may provide meaningful information concerning the biological situation in the vicinity of the outcrop where the spring occurs, but are unlikely to accurately reflect microbial activity deeper within the aquifer formation.

Well waters are considered to provide more representative information concerning subsurface ecosystems than spring waters, but they are likely to reflect contamination resulting from drilling operations, and they may not be quantitatively indicative of the microbial population in an aquifer since the movement of water from the surrounding aquifer rocks into a well during pumping is likely to be much more rapid than the movement of microbes. Samples obtained aseptically from undisturbed cores undoubtedly provide the most representative information concerning subsurface microbial activity, but the acquisition of such samples is fraught with difficulties, as discussed in more detail below. Also, since subsurface strata are not homogeneous, both cores and water from a single well may not accurately reflect the microbial population of the adjacent rock formation.

Well drilling operations alter and contaminate the subsurface environment, especially with wells of any depth because of the need for circulating drilling fluids or muds to remove the cuttings and keep the walls of the borehole from collapsing Drilling fluids often contain various sorts of additives and are a definite source of bacterial contamination.[46,58]

The possibility that surface microbes can be introduced during drilling and sampling operations has caused many to question whether microbes isolated from subsurface samples were actually native to that environment. This has often been a valid question, particularly where samples were obtained from old boreholes or producing wells, since such samples are hardly representative of nondisturbed, uncontaminated formations.

Despite the possibility of contamination, however, many workers are convinced that uncontaminated samples can be and have been obtained from subsurface regions and, hence, microbes isolated from such samples are indeed native to the subsurface formations sampled. These convictions are based on several observations and lines of reasoning. For example, as Davis points out, organisms necessarily were present at some time prior to drilling in some formations that have been studied because surface microbes introduced during drilling would not have had time to produce the biogenic products noted in these formations immediately after drilling.[46] Also, surface contamination could not have produced during the short time of drilling and coring the large populations of bacteria capable of utilizing hydrocarbons under subsurface conditions observed in some formations.[8] Others have suggested that contaminating surface microbes introduced into subsurface regions during drilling would perish at great depths due to a lack of adaptation to high pressures and temperatures; but, as indicated in the preceding section on the subsurface environment, this reasoning is not likely to apply to studies at most depths.[37,59] ZoBell, who has devoted considerable attention to the problems of sampling and contamination of samples from subsurface regions, has noted that many cores have been obtained in which no bacteria were found, thus indicating that the sampling techniques employed were capable of producing uncontaminated samples.[18]

A full-scale field and laboratory investigation to actually determine whether uncontaminated cores could be obtained by drilling operations was conducted in the Soviet Union in 1948 and 1955.[58] It was found that drilling muds were definitely a potential source of contamination, containing a variety of bacteria, including sulfate-reducers and hydrocarbon utilizers. To determine the range of penetration of bacteria from drilling muds into various types of cores, a series of special experiments were carried out. Circulating drilling fluids were seeded with a culture of Bacterium prodigiosum (Serratia marcescens), a bacterium which produces a unique red pigment when cultured. The seeded drilling fluid was introduced and circulated in the drill holes for 15-20 minutes before pulling the cores. The cores were then carefully examined layer by layer for penetration by the test organisms. Some representative results obtained in this study are presented in Table 3. As would be expected, it was found that the more porous a rock the greater the possibility that the core has been contaminated by the drilling fluid, since test bacteria penetrated throughout cores of sand and sand layers, but barely penetrated hard, compact rocks such as limestone. These studies emphasize the need for exercising great care in sampling subsurface

Table 3. DEPTH OF PENETRATION OF A BACTERIAL TRACER (Serratia marcescens) FROM DRILLING MUDS INTO DRILL CORES [From Smirnova, 1957 [58]]

Depth of sample, meters/feet	Lithologic description of sample	Depth of penetration (mm) by S. marcescens 75 mm diameter core
308/1011	Dolomite, dense.	1.0
311-314.3/1020-1031	Limestone, dolomitic.	1.0-1.5
218-223.5/715-733	Sandstone, fine-grained, very firm with calcareous cement.	1.5-2.0
18.5/61	Sandstone, grayish-brown, compact dry.	2.0-3.0
18.0/59	Sand, argillaceous, blue, marly.	3.0
377-377.5/1237-1239	Dolomite, calcareous, strongly porous with crumpled bedding.	4.0
35.75/117	Clay, dense, micaceous.	5.0
45.5/149	Clay, soft, very micaceous.	5.0
4.0/13	Clay, strongly arenaceous, friable, micaceous, black sand in lower part.	6.5
12.9/42	Clay, dense, with sand fragments.	7.0
208-213/682-699	Clay, with layers of green-gray sand, micaceous	Entire thickness of core.
8.0/26	Sand, grayish-brown, laminated, with layers of white and reddish-brown coarse-grained sand in lower part.	Entire thickness of core.
29.15/96	Sand, grayish-brown, argillaceous, very friable.	Entire thickness of core.
32.0/105	Sandstone, dark brown, grayish-brown, friable sand in lower part.	Entire thickness of core.

environments for microbial activity, but indicate that uncontaminated cores can be obtained from many subsurface regions if reasonable precautions are taken.

If an uncontaminated sample can be obtained, determining the number, type, and activity of any organisms present is also extremely difficult. The technical problems involved in such determinations have been discussed in detail by a number of microbial ecologists who all point out that it is presently impossible to accurately enumerate the microbial population of any ecosystem and measure the extent of its activity in the in-situ environment.[16,43,60,61,62]

Originally, the basic approach in attempting to enumerate the organisms in a sample was to count them directly with a microscope or indirectly by inoculating different culture media and counting the organisms which grew. Both methods of enumeration are extremely inaccurate since it is usually impossible to differentiate between living, dormant, and dead cells by direct microscopic methods and because different species of organisms vary greatly in their ability to grow on selected culture media. Therefore, it must be understood that microbes found in a sample by such approaches may not give a true indication of the total microbial community existing in the sampled habitat and the processes occurring there. However, the repeated presence of certain microorganisms in samples from the same kind of habitat is highly indicative that they are native to that habitat and their metabolic capabilities provide some idea of their role there and the existing conditions.

RESULTS OF INVESTIGATIONS OF SUBSURFACE MICROBIAL ACTIVITY

Early microbiologists generally considered the deeper portions of the earth's crust to be void of life until the 1920s when Bastin in the United States and Ginsburg-Karagitscheva in the Soviet Union reported the first credible discoveries of sulfate reducers and other bacteria in oil-field waters from depths as great as 1,000 meters (3,280 ft).[63,64,65,66] Although both investigators primarily sampled producing oil wells and, therefore, the organisms they found could have been contaminants, their reports apparently were the impetus to increased research in petroleum microbiology in both countries, a development which eventually expanded to include other phases of subsurface biology. Table 4 is a chronological listing of representative investigations

Table 4. REPRESENTATIVE REPORTS OF ORGANISMS ISOLATED FROM SUBSURFACE ROCKS AND WATERS

Main organisms isolated	Source of sample	Reference
Sulfate reducers	Oil-field waters from producing wells.	Bastin, 1926[63]
Sulfate reducers	Oil-field waters from producing wells.	Bastin, 1926[64]
Sulfate reducers	Oil-field waters from producing wells.	Bastin and Greer, 1930[65]
Sulfate reducers, denitrifiers, various other anaerobes	Waters from oil wells and sulphurous springs from depths as great as 1,000 meters (3,280 feet).	Ginsburg-Karagitscheva, 1933[66]
Pigmented sulfur-bacteria, sulfate reducers, denitrifiers	Oil-field waters from artesian wells.	Issatchenko, 1940[67]
Sulfate reducers	Drill-cores of sulfur-bearing limestone and anhydrite from a depth of 476 meters (1,560 feet).	ZoBell, 1944[68]

Table 4. REPRESENTATIVE REPORTS OF ORGANISMS ISOLATED FROM SUBSURFACE ROCKS AND WATERS (Continued)

Main organisms isolated	Source of sample	Reference
Anaerobes (sulfate reducers included)	Drill cores to a depth of 54 meters (177 feet).	ZoBell, 1949[69]
Sulfate reducers	Drill cores of sulfur-producing salt domes from depths of 213-274 meters (700-900 feet).	Miller, 1949[70]
Sulfate reducers	Drill cores of oil-bearing rocks from depths of 1,322-1,345 meters (4,337-4,412 feet).	ZoBell, 1951[71]
0-117x10^6 bacteria/gm of rock (direct counts)	Sedimentary rocks from depths as deep as 2,093 meters (6,887 feet).	Ekzertsev, V. A., 1951[72]
Sulfate reducers	Cores of sulfur-limestone-anhydrite and oil-bearing rocks from depths of nearly 3,050 meters (10,000 feet).	ZoBell, 1958[18]
0-366x10^3 bacteria/ml oil (direct counts)	Oil samples from depths of 238-2,296 meters (781-7,533 feet).	Meskov, 1958[73]

Table 4. REPRESENTATIVE REPORTS OF ORGANISMS ISOLATED FROM SUBSURFACE ROCKS AND WATERS (Continued)

Main organisms isolated	Source of sample	Reference
Diplococci	Salt crystals.	Reiser and Tasch, 1960[74]
Five species of Pseudomonas and Bacterium capable of oxidizing naphthalene	Underground waters from both oil and non-oil bearing regions.	Naumova, 1960[75]
Members of the genera Pseudomonas, Mycobacterium, and Pseudobacterium	Underground waters and cores from gas-bearing regions.	Telegina, 1961[76]
Eight species belonging to the genera Pseudomonas, Bacterium, Pseudobacterium, and Mycobacterium	Underground waters from oil- and gas-bearing regions.	Smirnova, 1961[77]
Saphrophytes, denitrifiers, sulfate reducers, heptane oxidizers, others	Ground waters from recharge zones, the vicinity of oil deposits and at discharge zones of several aquifers.	Al'tovskii, et al., 1961[37]

Table 4. REPRESENTATIVE REPORTS OF ORGANISMS ISOLATED FROM SUBSURFACE ROCKS AND WATERS (Continued)

Main organisms isolated	Source of sample	Reference
Sulfate reducers, sulfur oxidizers	Sulfur deposits.	Ivanov, 1962[78]
Sulfate reducers, others	Oil, water, and oil-bearing sand taken directly from a fractured oil bed.	Andreevskii, 1962[79]
Sulfate reducers, methane formers, other anaerobes	Water and oil from old and freshly-drilled oil deposits.	Sazonova, 1962[80]
Sulfate reducers, sulfur oxidizers putrefiers	Ground waters from springs and oil and gas wells.	Kuznetsova, 1962[81]
(Not named)	Rocks from drill holes.	Messineva, 1962[82]
Sulfate reducers, denitrifiers, methane formers, putrefiers, sulfur oxidizers, others	Ground waters from various strata.	Al'tovskii, 1962[83]
Hydrocarbon oxidizers	Ground waters from wells (pumped, artesian, dug).	Telegina, 1962[84]

Table 4. REPRESENTATIVE REPORTS OF ORGANISMS ISOLATED FROM SUBSURFACE ROCKS AND WATERS (Continued)

Main organisms isolated	Source of sample	Reference
Saphrophytes, denitrifiers, sulfate reducers, methane formers, others	Ground waters from various Paleozoic strata, both oil and non-oil bearing regions.	Mekhtiyeva, 1962[85]
A *Bacillus* species	Paleozoic salt deposits.	Dombrowskii, 1963[86]
Seven species of *Pseudomonas*, *Mycobacterium*, and *Chromobacterium* capable of oxidizing hydrocarbons	Waters of oil and gas beds.	Slavnina, 1965[87]
19 cultures of *Mycobacterium* capable of utilizing petroleum compounds	Waters underlying petroleum deposits.	Norenkova, 1966[88]
Hydrocarbon-oxidizing bacteria; methane, H_2, H_2S, N_2 producers	Cores and waters from tertiary deposits.	Bogdanova, 1966[89]
Complex of sulfate reducers and various *Pseudomonas* spp., others	Petroleum well.	Kuznetsova and Gorlenko, 1966[90]

Table 4. REPRESENTATIVE REPORTS OF ORGANISMS ISOLATED FROM SUBSURFACE ROCKS AND WATERS (Continued)

Main organisms isolated	Source of sample	Reference
Sulfate reducers, hydrocarbon oxidizers, hydrogen producers, butyric acid bacteria, others	Waters of oil and gas deposits from depths as great as 2,900 meters (9,514 feet).	Zinger, 1967[91]
Various groups of sulfate reducers Thiobacillus sp., oxidizers	Waters from wells in zones of recharge, submerged strata, and discharge.	Kuznetsova, 1967[92]
Spore formers, total heterotrophs	Shallow cores below the root zone (from below corrals, nonirrigated fields, sod).	Stewart, et al., 1967[38]
Sulfate reducers, sulfur oxidizers	Sulfur-bearing and sulfur-calcite rocks from a sulfur deposit beneath the ground-water level.	Pomerants and Belonitskaya, 1970[93]

made since the 1920s pertaining to microbial activity in subsurface environments. These investigations clearly show that a diverse microflora is found in various subsurface regions.

Initially, investigators examined oil-field waters for the mere presence of microorganisms with little thought being given to the possibility that the organisms they found were sampling contaminants. With time greater efforts were expended to determine whether microbes found in subsurface samples were actually native to the sampled regions or were introduced during the drilling or sampling procedures. Perhaps the most elegant and extensive effort was that of ZoBell, Sisler, and personnel of the Shell Oil Company which involved an attempt to drill an entire oil-well aseptically in order to determine whether the bacterial corrosion of oil-well casings at depths of several thousand feet in a California oil field were caused by bacteria native to those depths.[71] To minimize microbial contamination, an alkaline (pH 11.5) drilling mud was used, equipment was sterilized by means of germicides and heat produced by blowtorches, and special core-handling techniques were employed. The precautions taken were considered to be successful since no surface-type aerobic heterotrophs were found in cores. Viable sulfate-reducing bacteria were isolated from oil-bearing rocks taken from depths of 1,322-1,345 meters (4,337-4,412 feet).

Sulfate-reducing bacteria were the only microorganisms isolated in many initial studies because they were the only microbes which the experiments were designed to detect. Eventually, investigators examined the subsurface for other microbial groups and found a biochemically diverse microflora existing not only near petroleum and sulfur deposits, but also elsewhere in the subsurface. Various denitrifiers, methane formers, sulfur oxidizers, and hydrocarbon utilizers have been isolated, including members of the genera Thiobacillus, Pseudomonas, Methanomonas, Mycobacterium, Actinomyces, and Pseudobacterium. Although determinations of all numbers are inaccurate and undoubtedly do not reflect actual population densities in the in-situ environment, counts of several million bacteria per milliliter have been reported in some samples.

That sulfate reducers are not the only microbial group native to subsurface environments is illustrated by a study in which the pattern of distribution of hydrocarbon-oxidizing and gas-producing microflora within Pliocene and Paleocene deposits

of the USSR was investigated.[89] Although both core and water samples were examined, the results from cores are most significant because the outer layer of each core was carefully pared off and only the theoretically uncontaminated interior of the core was examined. Hydrocarbon-oxidizing, sulfate-reducing, and methane-producing bacteria were found in cores of various types of sandstones, marls, and clays mixed with limestone, siltstone, and argillite from depths of 87-504 meters (285-1,654 feet). No apparent relationship between the distribution of various bacteria and depth was observed, and different types of microbes were invariably found in the same core sample. Only one of twenty-nine cores examined did not contain any microbes which could be detected by the methods employed.

The presence in subsurface environments of nearly all the organisms which have been reported as residing in such regions is not contraindicated by available knowledge concerning their physiological capabilities. Reviews on the ecology of sulfate-reducing bacteria point out that sulfate reducers, primarily the vibrio Desulfovibrio desulfuricans, are uniquely adapted to grow in subsurface environments, and it would not be surprising if they were almost ubiquitious in such regions.[8,18,39,46,94] Sulfate reducers can tolerate an extreme range in osmotic and hydrostatic pressure, temperature, and pH. As a class they utilize a number of organic compounds such as various fatty acids, amino acids, simple alcohols, carbohydrates, and possibly some hydrocarbons. They may use elemental hydrogen. Oxygen is not required as a terminal electron acceptor since they are able to reduce a variety of sulfur compounds (sulfates, sulfites, thiosulfates, hyposulfites, and sulfur) to hydrogen sulfide (H_2S), which they tolerate at levels normally toxic for microorganisms. Sulfate-reducing bacteria are often found in association with other bacteria, a relationship which may be mutually beneficial [8,18,46] Associated organisms evidently are able to oxidize organic substances unusable by sulfate reducers, producing compounds which sulfate reducers can subsequently utilize as nutrients, with redox conditions conducive to growth of sulfate reducers being created during the process. Conversely, sulfate reducers may provide sulfur for associated organisms in a form more utilizable than sulfate. The pumping of large quantities of fresh water into formations containing organic deposits is thought by some to stimulate eventual sulfate reduction by the development of an aerobic, organic-oxidizing microflora and the resultant production of

nutrients usable by sulfate reducers.[90,95] Ironically, sulfate reducers evidently are adversely affected by conditions such as high temperatures not directly inhibitory to them but which are inhibitory to associated organisms.[90]

In general, bacteria such as Pseudomonas, Mycobacterium, and Actinomyces species found in subsurface samples are also commonly found in upper soil layers. They are capable of growth in anaerobic environments by using nitrate or organic compounds as electron acceptors instead of oxygen. As a group they utilize a wide range of organic compounds as nutrients. A number of different species have been isolated from subsurface samples which in laboratory studies could oxidize hydrocarbons ranging from napthalene to alkanes containing from one to ten carbons.[75,76,77,87,88,96] The number of compounds that organisms such as various Pseudomonas species are able to attack and degrade is vast, although they may be able to attack many substances only under aerobic conditions.

The presence in subsurface environments of some organisms reported to have been isolated from subsurface samples has been questioned because such organisms seemed to be unsuited for the conditions theoretically present in those environments. For example, some early Russian investigators reported the isolation of purple sulfur bacteria from stratal waters of some oil deposits.[67] Since such bacteria cannot grow in the dark without oxygen their presence in deep subsurface waters seemed unlikely. It is now believed that they were introduced into the oil strata via injection wells and were able to survive by using the oxygen contained in the air and water pumped into the well and by utilizing the byproducts of hydrocarbon-oxidizing bacteria also present in the strata.[97]

Another seeming contradiction has been the isolation of both sulfate reducers and sulfur oxidizers from the same samples. However, Thiobacillus denitrificans, one of the sulfur bacteria, is a facultative anaerobe capable of using nitrate instead of oxygen as an electron acceptor while oxidizing H_2S, sulfur, or thiosulfate to sulfate.[98] It may be possible for such organisms to live in association with sulfate reducers, although this has not been examined under controlled experimental conditions.

It is impossible to determine how long microorganisms have been in subterranean formations where they are found, especially since microorganisms isolated from such formations do not differ from surface microbes to any obvious

extent. The microorganisms found in deep stratal waters may have been deposited with the sediments millions of years ago or they may have migrated into such strata from the surface over an undetermined period of time.[18,37,74,82,86] Bacteria are not only carried by underground waters moving through fractures, fissures, channels, solution cavities, and pore spaces, but they are also often able to propel themselves through water, perhaps to reach better conditions or to escape unfavorable environments. Such movement is extremely slow, ranging from a few microns to a few centimeters per day.[14] It is possible that organisms could migrate outward from drilled wells over a period of years, contributing to colonization of some formations, since proliferation of microbes in the immediate vicinity of wells often causes severe corrosion and clogging problems.[46,99,100,101] The extent to which such growth radiates from wells is uncertain, however. Studies on the movement and growth of microorganisms in aquifers near wells have pertained mainly to tracing the movement of coliform bacteria, various pathogens, or other nonnative microbes over relatively short periods of time.[7,102,103] As noted previously, the limited migration of such microbes in subsurface regions is likely due in part to their limited ability to survive and proliferate in such environments. The outward migration over a significant period of time of bacteria more suited to subsurface conditions has apparently not been investigated to any extent.

The broad spectrum of environmental possibilities in subsurface regions obviously affect in many ways the distribution and activity of microorganisms in subsurface regions. There have been several studies in which the chemical and physical conditions in various subsurface regions were compared with the microflora found there in an attempt to determine the effect of various conditions. Some of the first studies embodying this approach were begun by Soviet workers in the 1950s who attempted to relate the organic, inorganic, and bacterial composition of several oil-bearing aquifers to the origin of petroleum in these aquifers.[37,81] To do so they compared the bacterial and chemical compositions of subsurface waters from three distinctly different zones in each oil-bearing aquifer. These zones were (1) the zones where the aquifers were recharged, (2) the zones where the moving waters were associated with oil and gas deposits (anticlinal traps), and (3) the zones where the waters were discharged to the surface. They found that the microflora of various zones

within the same aquifer differed sharply, the differences apparently being related to the temperature of the ground waters, the oxidation-reduction potential, and the content of organic material, oxygen, and sulfate. This is best illustrated by Kuznetsova's data on ground waters of the Grozny-Dagestan province in the USSR (Table 5).[81] Waters with low temperatures (<50°C), high oxidation-reduction potentials, and oxygen contained primarily "putrefying" bacteria ranging in numbers from 59,000 to 1,000,000 per milliliter. Waters with high temperatures (>50°C) and lowered oxygen and oxidation-reduction values contained greater quantities of H_2S indicating the activity of sulfate reducers. Temperatures greater than 50°C resulted in considerably reduced counts. Sulfur-oxidizing bacteria were abundant when the oxidation-reduction potential of the water was between rH_2 values of 11.6-21.

Mektiyeva investigated the microflora in ground waters of Paleozoic strata and compared the distribution of microbes in relation to the water's contact with petroleum.[85] She found that waters which were not in direct contact with petroleum or gas deposits still contained an active microflora, although not as abundant and varied as the microflora of waters in contact with petroleum deposits. Although the number of bacteria decreased with depth, the distribution of the microorganisms was related to the depths they were found only to the extent that the strata at that depth affected the chemical composition of the ground waters. For example, it was felt that the microflora in some samples obtained from deep formations was limited not because of the depth, but because the waters from that strata were highly saline, lacked sulfates and other electron acceptors, had high Cl-Na/Mg ratios, and contained up to 1400 ppm bromine.

A study along the same lines as that of Russian workers was conducted by the Socony Mobil Oil Company on a large oil-bearing fresh-water aquifer in the Carrizo Formation in south Texas.[46] In this study an effort was made to correlate the organic, inorganic, and bacteriological characteristics of the ground waters of the aquifer with the presence or absence of oil. Nine wells, six of which were free-flowing, were sampled. These wells were spread along a line roughly 36 miles long which started with Well No. 1 above the oil field at the recharge area and

Table 5. COMPARISON OF THE AVERAGE BACTERIAL AND CHEMICAL COMPOSITION OF THREE DIFFERENT ZONES WITHIN OIL-BEARING AQUIFERS [From Kuznetsova[81]]

	Recharge zone	Oil deposit zone	Discharge zone (springs)
Temperature, °C	12-21	23-89	12-87
rH_2	17.8-30	9.7-17.8	1.6-15
O_2, mg/l	0.6-5.6	0	0
H_2S, mg/l	0-0.2	0-40	4-57
Direct microscopic count of bacteria per ml ($x10^3$):			
@ temperatures <50°C	59-1036	105-9600	144-3137
@ temperatures >50°C	-	25-72	9-40
Relative abundance of bacteria:			
Desulfurizing	+ or -	++	+++
Sulfur-Oxidizing	+ or -	++	++++
Putrefying	+++	+ or -	+

proceeded with increasing depth in the direction of subsurface water movement until the oil field was passed. The results of sampling are discussed in detail by Davis.[46] Some average results are shown in Table 6. The waters in this aquifer are fresh, having a total solids content of less than 800 ppm and a chloride content of less than 100 ppm. The water moves at a very fast rate of 50-100 feet per year. Bacterial plate counts of well samples were relatively low (<100,000 per liter). Aerobes were found consistently only in Well No. 1, near the recharge area. Sulfate reducers were found in all wells except No. 7 and 8. Their absence in these two wells could possibly be due to the high temperatures although sulfate reducers were found in Well No. 9 where the temperature was also high. Sulfur oxidizers were not found in any well. Facultative bacteria were most numerous in Wells No. 1 and 6 where the organic matter was the highest. It should be noted, however, that these two wells were pumped wells while the others checked for facultative bacteria were free-flowing; also, the analytical methods employed would detect only a part of the dissolved organic matter present and, hence, the data presented undoubtedly do not accurately reflect the total organic matter in these subsurface waters and rocks. Davis believes that the increase of sulfide and possibly the increase of ammonia was due to bacterial activity as the water moved through the formation.

Obviously, most investigations of subsurface biology have been concerned with the soil zone or with deeper regions of the zone of saturation. Very little information has yet been developed pertaining to biological activity in the upper regions of the zone of saturation and those regions of the zone of aeration lying below the soil water zone. One study which did include microbiological investigations of these regions was concerned with the distribution of nitrates and other pollutants under fields and corrals in the valley of the South Platte River in Colorado.[38] In this study, conducted by the United States Department of Agriculture, cores were taken in nonirrigated pastures, cultivated fields, and corrals at one-foot intervals down to the water table or bedrock, and the cores and water samples were subjected to various chemical and bacteriological analyses. Results from microbiological analyses showned that microbial activity continued below the root zone and that often there was a distinct increase in total counts at or immediately above the water table. A higher bacterial population was found

Table 6. SOME RESULTS OF BACTERIOLOGICAL AND CHEMICAL INVESTIGATIONS OF THE GROUND WATERS OF AN OIL-BEARING AQUIFER IN SOUTH TEXAS [After Davis [46]]

	Well No. 1	Well No. 2	Well No. 3	Well No. 4	Well No. 5	Well No. 6	Well No. 7	Well No. 8	Well No. 9
Type of well	Water well	Water well	Water well	Oil well	Water well	Oil well	Water well	Water well	Water well
Location to oil accumulation	Up-dip	Up-dip	Up-dip	-	-	-	Down-dip	Down-dip	Down-dip
Well flow	Pumped	Free-flowing	Free-flowing	Pumped	Free-flowing	Pumped	Free-flowing	Free-flowing	Free-flowing
Well depth, ft	215	1,137	1,800±	1,690	2,100	1,846	3,200	4,100	4,200
Temperature, °C	23	30	34.5	-	38.5	32	47	58	58
pH	6.6	6.9	7.2	8.6	7.3	8.5	8.1	8.3	8.5
Organic matter, μg/l[a]	28.7	2.9	2.6	-	-	4,000	15.0	8.3	11.5
Ammonium, ppm	0.09	0.05	0.1	0.	0.1	0.36	0.36	0.5	0.45
Sulfate, ppm	12.0	36.0	33.0	2.4	52.0	3.4	12.0	46.0	34.0
Sulfide, ppm	0	0	0	0	0	0	14.5	4.8	22.0
Sulfate-reducing bacteria/liter	1,000	1,200	7,000	-	-	1,000	0	0	10,000
Facultative bacteria/liter	110,000	-	360	-	-	900,000	4,000	0	20,000

[a]Includes only that organic matter obtained from water by carbon filter and recovered from the carbon by extraction with chloroform

below corrals than beneath fields, possibly because of a greater amount of available carbon. Nitrifying bacteria were not found to any great extent below the upper few feet. The investigators felt that denitrification was probably occurring in deeper regions of the zone of aeration under corrals since nitrate was absent and total bacteria counts were high. It should be noted that this study provides no information concerning the presence of obligate anaerobes since microbiological cultures were grown under aerobic conditions only. Also, the examined samples were initially frozen and kept for an unstated period of time until they were analyzed, which probably influenced the results obtained in the microbial studies to some extent.

SIGNIFICANCE OF PREVIOUS SUBSURFACE MICROBIAL STUDIES AND NEEDED ADDITIONAL INVESTIGATIONS

The information obtained in the previous investigations of subsurface microbial activity described above clearly illustrates the presence of a diverse microbial population in many regions of the earth's crust lying below the soil zone. This information, in conjunction with available information concerning the suitability of subsurface environments as microbial habits, indicates strongly that microbial activity is both possible and probable in most subsurface regions which are of importance in regard to ground water. The significance of such activity to ground-water pollution and pollution control, of course, resides in the potential interactions of pollutants and microorganisms in subsurface regions and the ultimate effect of such interactions on the quality and availability of ground water. Subsurface microbe-pollutant interactions are likely to be mainly beneficial, producing such desirable results as elimination of organic pollutants from subsurface waters by mineralization or removal of nitrate by denitrification. Such interactions might also sometimes be detrimental, resulting in production of undesirable metabolic products which enter and pollute ground water or reduction of aquifer permeability through clogging of interstitial space by cells and polymeric products of metabolism.

Previous investigations of subsurface microbial activity have been concerned primarily with delineation of the types and numbers of native microbes in essentially undisturbed subsurface environments, mostly in formations deep within the zone of saturation. These investigations provide a

limited indication of possible microbe-pollutant interactions which might be expected to occur in some subsurface environments. However, they provide practically no information concerning: (1) the extent and nature of native microbial activity in the zone of aeration below the soil zone and in the upper regions of the zone of saturation; and (2) microbial activity in subsurface environments altered by the introduction of pollutants.

Microbial activity in the zone of aeration below the soil and in the upper portions of the zone of aeration is obviously of very great potential importance in regard to groundwater pollution and pollution control. Systematic studies to define the extent and nature of native microbial activity in these regions, correlating microbial data with environmental characteristics, particularly geological and oxidation-reduction conditions, are needed. These studies would provide data which would be useful in preliminary efforts to predict the fate of pollutants in these regions of the earth's crust, and which would serve both as a basis and complement to more extensive investigations pertaining to the movement, fate, and effect of pollutants in subsurface waters.

Microbial populations, and hence microbe-pollutant interactions, in subsurface regions receiving pollutants are likely to be profoundly affected by the nature and quantity of pollutants introduced and their effect on the native subsurface environment. For example, microbial species which are very minor members of the native ecological community may become dominant in the environment created by entry of pollutants. Also, microbes indigenous to the pollutant-containing waste introduced into a subsurface region may proliferate there, becoming the dominant species and controlling the fate of pollutants entering that region.

The above observations indicate the need for thorough, integrated investigations of microbe-pollutant interactions in subsurface regions receiving pollutants. In-situ investigations should be employed where possible, but laboratory simulation work utilizing core samples would probably often be required, particularly in the study of deep formations. Such studies should seek to correlate native microbial populations, environmental situations (geology, oxygen and nutrient availability, pressure, temperature, etc.), nature of pollutants, and microbial populations subsequently developing after the introduction of pollutants, with the fate of pollutants and their effect on ground-water quality

and availability. Investigations of this type would necessarily be complex and expensive, but they would provide information needed in developing methods which would permit prediction of the probable impact on ground water of various activities entailing release of pollutants into the earth's crust, including waste disposal, agricultural, and ground-water recharge activities, and which, therefore, would provide the basis for logical regulation and control of such activities. Such studies might also indicate how microbial activity in subsurface regions below the soil zone could best be managed and utilized to achieve maximum degradation of pollutants in subsurface waters. For example, improved knowledge of the factors controlling subsurface microbial activity might permit alteration of subsurface environmental factors and/or microbial populations to bring about mineralization of hydrocarbons or other organic compounds accumulating in aquifers as the result of accidents involving transport or storage equipment, or to achieve beneficial alteration of wastes intentionally introduced into deep formations.

SECTION VI

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Biological activity occurring in subsurface regions below the soil zone may be of considerable importance in determining the fate and effect of pollutants in ground water, but this possibility has received little previous attention. This paper comprises a discussion of subsurface biological activity in regard to ground-water pollution as reflected by available literature references. The subsurface environment is discussed in terms of factors likely to be of greatest significance in regard to the development of biological systems, and previous investigations of subsurface microbial activity are reviewed. Available information indicates the presence in the upper continental crust of the earth of numerous regions, particularly those of sedimentary origin, which are probably suitable habitats for many microbial species. Previous investigations of subsurface microbial activity clearly show the presence of diverse microbial populations in many subsurface regions below the soil zone. Hence, microbial activity appears both possible and probable in most subsurface regions of importance in regard to ground water. Further elucidation of the extent and nature of microbial activity in subsurface regions is needed in developing methods for predicting the impact of pollutants on ground-water quality.

17a. Descriptors

*Ground water, *Subsurface investigations, *Microbiology, *Subsurface waters, *Habitats, Water pollution, Water pollution effects, Path of pollutants, Zone of aeration, Zone of saturation, Biological communities, Biodegradation, Temperature, Hydrostatic pressure, Anaerobic bacteria.

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